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13. ABSTRACT (Maximum 200 Words)

In this proposal, we hypothesize that inhibition of IGF action by IGFBP-1 will prevent breast cancer in a SV40 Tag transgenic model of breast cancer. We will test this hypothesis with two specific aims: 1) to inhibit IGF action at the mammary epithelial cell by creating transgenic mice that express IGFBP-1 under the control of the whey acidic protein (WAP) promoter and 2) to test the ability of IGFBP-1 to suppress tumorigenesis by mating these animals with C3/Tag transgenic mice. To date, we have generated two founder lines containing the IGFBP-1 transgene and several F1 and F2 animals were analyzed. Unfortunately, while the transgene was clearly integrated into these animals, we were unable to detect expression of IGFBP-1 protein. To correct this problem we have generated more founders with a modified construct involving insulator sequences. Oocytes have been injected and we are awaiting the offspring.

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Introduction
The purpose of this project was to create transgenic mice expressing IGFBP-1 in the mammary gland. We hope overexpression of this binding protein can neutralize IGF action and inhibit breast cancer development.

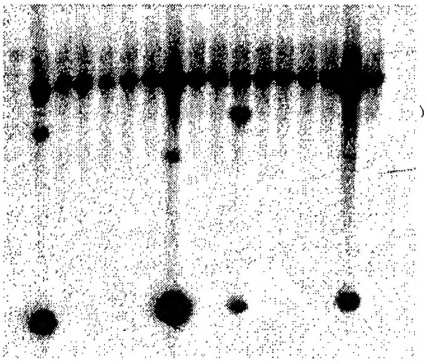
Body
Specific Aim (Task) #1 - To inhibit IGF action at the mammary epithelial cell by creating transgenic mice that express IGFBP-1 under the control of the whey acidic protein (WAP) promoter

a. Months 0-3 - Create WAP-IGFBP-1 transgene vector
b. Months 3-9 - Create and identify IGFBP-1 F1 progeny
c. Months 9-16 - Characterize level of IGFBP-1 expression in mammary gland, determine influence of IGFBP-1 expression on lactation, examine activation of IGFR1

As noted in last year's progress report, we showed that we were able to identify integration of the transgene, but were not able to identify F1 generation animals with mRNA or protein expression of IGFBP-1. In last year's report, we felt that we selected too few animals.

In order to address this concern, we decided to re-inject animals with the original WAP-IGFBP-1 vector. Our collaborator (Dr. David Largaespada) reviewed our progress and suggested that addition of an insulator sequence may potentially enhance the ability of the transgene to be expressed. With Dr. Largaespada's assistance, we created a new transgene construct bearing the insulator sequence.

While we generating the new construct in preparation for oocyte injection, our specific pathogen free (SPF) animal facility suffered an unfortunate outbreak of pinworms and mouse hepatitis virus. Our Animal Use Committee decided to completely close the facility, transfer the animals to the animal facility in a newly constructed building, sterilize the old facility, then re-open the transgenic core facility for investigator use.



These outbreaks in the SPF facility essentially delayed this work for 12 months as the transgenic facility was closed for that period of time. Obviously, this contamination of the mouse facilities has significantly delayed our progress on this project.

Despite this setback, we were able to derive new founder animals. Figure 1 shows Southern blots of several of the separate founder lines we are now analyzing. We currently have 17 separate founder lines, a substantially increased number of animals compared to last year's progress report. We are in the progress of generating F1 animals and analyzing them for protein and mRNA expression.

Figure 1 – Southern Blot of Founders

Key Research Accomplishments
- Generated insulator constructs and injected into oocytes
- Generated 17 founder animals

Reportable outcomes
Given the necessity to generate new founder animals, we do not yet have reportable outcomes.

Conclusions
The progress in this proposal has been unfortunately delayed. Our initial oocyte injections yielded only a small number of animals who had integrated the transgene, but did not express IGFBP-1 mRNA or protein.

Unfortunately, we were unable to re-inject oocytes for nearly a year because of an infectious outbreak in our animal SPF facility. This has now been rectified, and we are back on track regarding generation of founders. We have been granted a no-cost extension and hope to complete the original aims of the proposal within the upcoming year..

References – None

Appendices - None